# Production of Extracellular Material By Jensen Sarcoma Cells *in Vitro.* (27017)

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Acid mucopolysaccharides and fibers characteristic of connective tissue have been produced *in vitro* by fibroblasts from mature and embryonal tissues(1-5). Mouse lung cells in young cultures were reported to produce collagenase sensitive fibers(6), but this capacity was lost after successive subculture. Abdel-Samie *et al.*(7) found HeLa cells and HLM cells produced species specific albumin in tissue culture. According to Teyssie *et al.*(8), Sarcoma 180 fibroblasts *in vitro* did not produce mucopolysaccharides in quantities detectable by histochemical methods, but small quantities of mucopolysaccharides were demonstrated in the nutritive media by turbidimetric methods. The current study demonstrates the ability of the Jensen sarcoma cells to produce quantities of extracellular material.

*Methods.* Jensen sarcoma cells were grown in a continuous perfusion system utilizing glass helices as a matrix upon which the cells would adhere. The initial inoculum was 400,000 cells/ml, and the medium used was Medium 5a(9). The initial perfusion rate was 70 ml/24 hrs and subsequent increases in this rate were determined by glucose utilization and pH of the effluent medium. After 9 days of growth the tissue was clearly visible to the naked eye and the cells were harvested. The glass helices with the adherent cell clumps were placed in Bouin's solution at room temperature for 24 hrs. The Bouin's solution was then decanted, and the cell clumps were separated from the helices by agitation in 70% isopropanol. The helices were allowed to settle, and the supernatant containing the cells and extracellular material was removed. The cell aggregates were then further dehydrated through a graded series of isopropanol and embedded in paraffin. Three and 5 $\mu$ sections were stained with hematoxylin and eosin, Malloy's trichrome, Lhotka's periodic acid-foot variant(10), Sudan black B, and PAS with diastase digested controls. The methylene blue extinction test, and ninhydrin-Schiff reactions were performed according to the methods outlined by Pearse(11). Other sections were treated respectively with bovine testicular hyaluronidase and streptococcal (chondroitin sulfatase free) hyaluronidase*, 1 mg/ml in phosphate buffer at pH 6.8 for 12 hrs at 37°C. Control sections were incubated in buffer alone. The hyaluronidase treated sections along with their controls were then stained with 1% Alcian Blue in 3% acetic acid(12), Muller's colloidal iron according to the method of Mowry(13), and Toluidine Blue after sulfation(14).

*Results and Discussion.* The comparison of the Jensen cells as they appeared in stationary T-15 cultures, the perfusion system, and *in vivo* can be seen in Fig. 1. The cells in the perfusion chamber more closely resemble those grown *in vivo* than do the cells cultured in T-15 flasks. They tended to mold to the contour of adjacent cells, overgrow the helices, and form a cloudy gelatinous mass. The consistency of the mass is due in part to extracellular material of 2 types—one consisting of amorphous masses and the other intimately related to individual cells. These differ histochemically.

The amorphous material (Fig. 2), stained with H and E, Mallory trichrome, and reticulum stains, failed to exhibit fibers. This substance stained faintly with PAS and exhibited a sulfation induced metachromasia. These staining reactions were not altered by incubation for 3, 12, and 20 hrs with testicular hyaluronidase or streptococcal hyaluronidase. The staining reaction with PAS was diastase fast. The material did not stain with Sudan B, thereby eliminating sphingolipids; and it did not stain with Alcian Blue or colloidal iron, precluding the possibility of a mucopolysaccharide. The methylene blue extinction was above pH 4, and the ninhydrin-Schiff reaction

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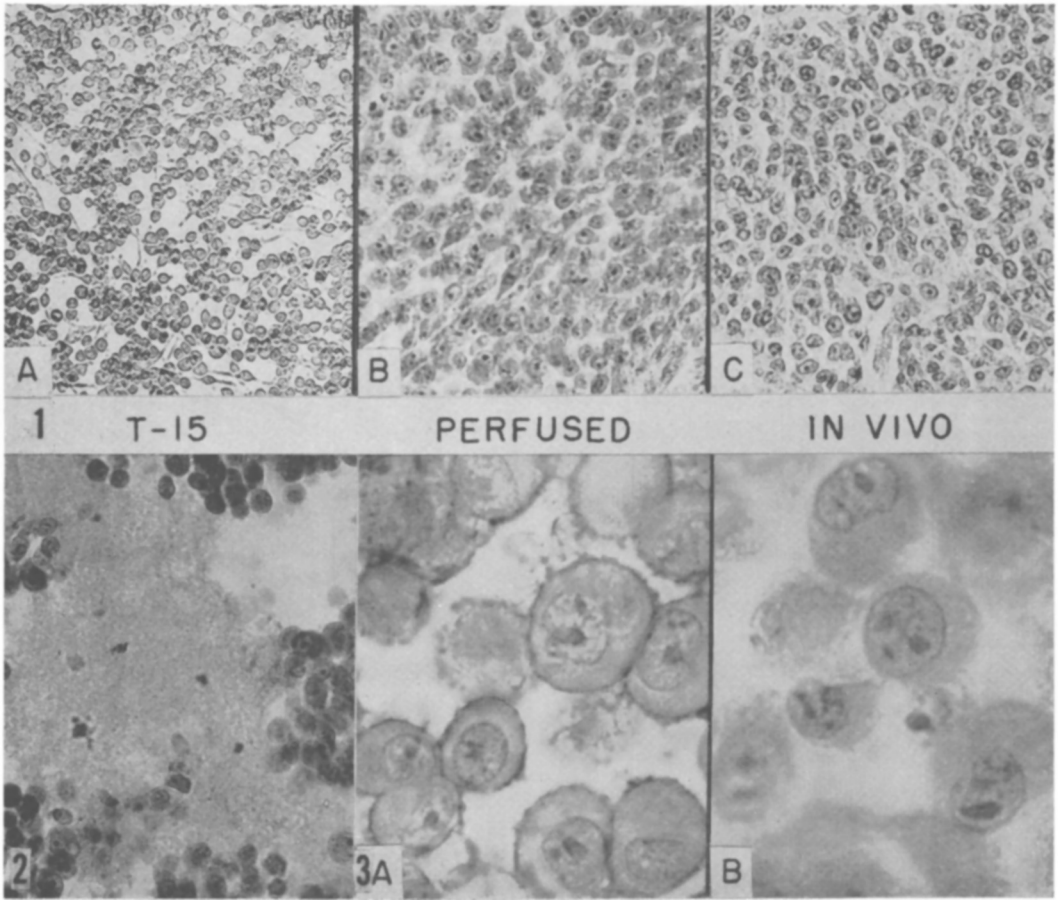


FIG. 1. Comparison of growth patterns of Jensen sarcoma cells grown (A) in the T-15 flask, (B) in a perfusion system, (C) *in vivo*. Note similarity of B and C. (A) Unstained. (B) and (C) Hemotoxylin-Eosin. x112.

FIG. 2. Jensen sarcoma grown in a perfusion system showing masses of amorphous extracellular material. PAS-Hematoxylin. x125.

FIG. 3. Sections of cultures of Jensen sarcoma (A) before and (B) after treatment with streptococcal (chondroitin sulfatase free) hyaluronidase. Colloidal iron-Hematoxylin. (A) - x 840; (B) - x 1120.

was positive. These data suggested the material was a muco- or glycoprotein, a simple protein with serum albumin adsorbed from the media, or a mixture of these substances.

The second type of material which was found in close association with individual cells stained brilliantly with Alcian Blue and with colloidal iron. These staining reactions were removed by 12 hrs incubation with streptococcal hyaluronidase (Fig. 3). This material, therefore, was hyaluronic acid and not chondroitin sulfate.

It is possible that the Jensen sarcoma cells produce small quantities of these materials in T-15 flasks, but it is not readily demonstrable because of the small amount of cellular material produced. A similar situation was reported to occur in Sarcoma 180 cells(8). The Jensen sarcoma cells, unlike some neoplasms, apparently do not produce hyaluronidase(15); therefore, hyaluronic acid must accumulate around the cells.

*Summary.* Jensen cells grown in a continuous perfusion system produce extracellular material of two types. One is a muco- or glycoprotein or possibly a simple protein; the other, hyaluronic acid.

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# Kidney Enzymes of Gluconeogenesis, Glycogenesis, Glycolysis and Direct Oxidation.* (27018)

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Studies of the past two decades demonstrated that the kidney carries out manifold metabolic functions of primary physiologic importance in addition to urine production. The various metabolic contributions of the kidney have been reviewed(1). One of the striking properties of kidney, shared only by liver, is its capacity to produce sugar. Earlier studies involving glucose measurement in arterial and renal vein and recent work utilizing kidney slice incubation techniques to demonstrate net synthesis of carbohydrate were discussed(1,2). More recent investigations on the alternate pathways of glucose metabolism of kidney employed labeled glucose or pyruvate and conclusions were reached on the behavior of metabolic pathways by determination of metabolites and end products of the various pathways(1,2). Thus, it has become of interest to obtain detailed information regarding the activities and behavior of the enzyme systems involved in glucose metabolism in this tissue.

The present work describes the carbohydrate enzymes involved in gluconeogenesis, glucose production, glycogenesis, glycolysis and direct oxidative pathway in the kidney. The enzymes studied are responsible for channeling glucose-6-phosphate into four alternate pathways: glucose-6-phosphatase (glucose release); phosphoglucomutase (glycogen synthesis); phosphohexose isomerase (glycolysis); and glucose-6-phosphate and 6-phosphogluconate dehydrogenases (direct oxidative pathway). The activities of other enzymes of gluconeogenesis and glycolysis (fructose-1,6-diphosphatase and lactic dehydrogenase) are also given. These carbohydrate metabolizing enzymes were studied in the kidney cortex of rats and values were compared with activities found in the liver. The investigation revealed a distinct enzyme pattern in kidney cortex which can be correlated with the behavior of metabolic pathways in this organ as elucidated by isotope approaches.

*Materials and methods.* Male Wistar rats of 250 g of weight were maintained on Purina Laboratory Chow and water *ad libitum*. The animals were stunned, decapitated and exsanguinated. Kidneys were quickly removed,

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